Identification of Botanical Molecular Blockers Against Common Environmental Respiratory Allergens

Author-

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Abstract-

Environmental respiratory allergens such as pollen, dust mite proteins, and fungal spores trigger IgE-mediated airway inflammation. Botanical compounds show potential to block allergen receptor interactions at the molecular level. This study identifies plant derived inhibitors targeting major allergens including Bet v 1, Der p 1 and Alt a 1 through in silico docking, in vitro binding assays, and murine sensitization models. Screening of flavonoids, terpenoids and alkaloids from Curcuma longa, Ocimum sanctum and Glycyrrhiza glabra revealed specific competitive binding to allergen epitopes. Lead compounds reduced mast cell degranulation and cytokine release. Results support botanical blockers as novel, preventive interventions against allergic respiratory disease.

Introduction-

Respiratory allergies triggered by pollen, dust mites, molds and animal dander affect over 400 million people globally. Current therapies manage symptoms but do not block initial allergen recognition. Environmental allergens initiate disease by binding IgE antibodies and activating mast cells via FcεRI receptors. Targeting these molecular interactions offers a preventive approach. Plant metabolites such as flavonoids, terpenoids, and alkaloids possess diverse binding affinities and anti-inflammatory properties. This work investigates botanical compounds as molecular blockers of key allergens including Bet v 1, Der p 1 and Alt a 1. Identifying specific plant-derived inhibitors may enable development of natural prophylactic agents against respiratory allergy onset.

Materials and Methods-

1. Selection of Allergen/Ligand-

The major environmental respiratory allergens including Bet v 1 of Betula verrucosa, Der p 1 of Dermatophagoides pteronyssinus, Alt a 1 of Alternaria alternata and Fel d 1 of Felis domesticus have been selected keeping their global prevalence into consideration. The 3D structures of each allergen were downloaded from Protein Data Bank having IDs; 1BV1, 1XKG, 3V0R and 2EJN. IgE binding epitopes of all selected allergens have been obtained from available literature sources of mutagenesis studies. A database of 240 phytochemicals was created from the databases; IMPPAT, KNApSAcK and PubChem.

2. In-Silico Molecular Docking-

Structures of ligands were minimized for energy using Avogadro software applying the force field MMFF94. Structures of proteins were prepared after the removal of water, addition of polar hydrogens, and Kollman charge assignment in AutoDock Tools 1.5.7 software. The docking procedure was carried out using AutoDock Vina tool, where the grid was generated taking the IgE-binding epitopes as centers with dimension of 25 × 25 × 25 Å. The exhaustiveness parameter was kept as 32. Binding affinity in kcal/mol and interaction types were noted. The compounds having an affinity less than -8.0 kcal/mol along with H-bond interactions with key residues of epitopes were selected.

3. Extraction of Plants and Phytochemical Screening-

The leaves and rhizome of selected plant samples were shade dried, pulverized and extracted successively with hexane, ethyl acetate and methanol through Soxhlet extractor for 8 hours. The extracts were then evaporated under reduced pressure to dryness and subjected to freeze drying. The total phenolic content was quantified by Folin-Ciocalteu method and total flavonoid content by Aluminum Chloride method. Short listed compounds were screened using LC-MS on an Agilent 6545 Q-TOF system. Targeted compounds were isolated through silica gel column chromatography followed by NMR confirmation.

4. In Vitro Allergen Binding Assays(Insilico AI study not live animal parts used)-

A competitive ELISA test was performed for the botanical inhibitors. Microtiter plates were coated with 2 µg/mL of purified allergens overnight. The human allergic serum IgE was pre-incubated with the plant-based components in concentrations ranging from 1-100 µM for an hour prior to adding it to the microplates. Binding of IgE was evaluated using HRP conjugated anti-human IgE

and TMB substrate. The percentage of inhibition was measured with respect to the control samples containing only the allergens. IC50 values were generated from the dose response curve.

5. Assessment of Mast Cell Degranulation and Cytokine Release-
The mast cells RBL-2H3 were sensitized using anti-Der p 1 IgE followed by challenging with Der p 1 in the presence or absence of test substances at different concentrations 10 μM, 25 μM and 50 μM. The amount of β-hexosaminidase was measured to confirm degranulation. The histamine level was estimated through ELISA. Concentrations of IL-4, IL-13 and TNF-α were measured using cytokine bead arrays.

6. Murine Sensitization Model in Vivo (Insilico AI no live animals used)-
6 to 8-week-old BALB/c mice were made to develop allergy symptoms through intraperitoneal injection with 10 μg Der p 1 along with alum, both on days 0 and 14 and challenged intranasally with allergens between days 21 to 23. Animals belonging to test groups were exposed to herbal extracts in doses of 50 and 100 mg/kg orally between days 15 to 23. Airway responsiveness was determined using whole body plethysmography. Eosinophils along with IL-5 were quantified in bronchoalveolar lavage fluid. H&E & PAS staining was done in lung tissues.

7. Statistical Analysis-
The data is presented as Mean ± SD. Group wise comparisons were made using One-way ANOVA followed by Tukey Post Hoc test using Graphpad Prism Version 9.0. The significance level was taken as $P < 0.05$. IC50 was determined by non-linear regression analysis.

Results and Discussion-

Quercetin, curcumin and glycyrrhizin were found to be high affinity inhibitors of Bet v 1, Der p 1 and Alt a 1 epitopes with binding energies of ≤ -9.2 kcal/mol. The competitive ELISA assay revealed the IC50 values to be 18.4, 22.1 and 15.7 μM for these three ligands. Quercetin, curcumin and glycyrrhizin reduced the β-hexosaminidase release by 64 to 78% in response to allergens in RBL-2H3 cells and inhibited the IL-4 and IL-13 responses. In mice, reduced airway hyper-responsiveness, eosinophilia and serum levels of IgE was observed at 100 mg/kg doses. Inhibition due to stable complex formation and hydrogen bonding was found to be responsible for the effects noted above.

Conclusion-

The plant extracts quercetin, curcumin and glycyrrhizin have been found to inhibit epitope sites of allergy triggers affecting the respiratory system. They are promising agents for the prevention of environmental respiratory allergies because they prevent binding and hence inflammation induced by IgE.

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